

7. The alumina is then placed under 200 ml distilled, deionized water and deaerated for at least one hour. The supernatant solution is then discarded.
8. The solution of step 5., above, is then added to the alumina of step 7., above, and the pH is adjusted to 4.2.
9. One-tenth gram EDC, i.e., 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride is added to step 8., above. After a half hour reaction period, the pH is adjusted to 5 with sodium hydroxide.
10. Four-tenths of a gram of EDC, is dissolved in 30 ml distilled, deionized water, and added to step 8., above at a rate of 1/10 ml per minute.
11. The solution of step 10., above is allowed to react for 12 to 16 hours, on the laboratory shaker, at 0° C.
12. The final product is washed and stored.
13. The activity of this preparation is measured as 24 Units glucose oxidase per milliliter alumina-glucose oxidase composite, in a 0.01 molar acetate buffer-substrate solution, pH 5.5, with no detectable amounts of catalase activity.

EXAMPLE 10

A continuous stream quantitative determination of the antigen phenobarbital in a sample may be accomplished using the apparatus according to the present invention. The apparatus shown in FIG. 2 is equipped with a solubilization stage 44, comprising a cartridge of approximately 8 ml total volume and a sample 46 of approximately 0.1 ml is injected into a flowing aqueous buffer stream. The stream contains 55 mM THAM buffer, [tris(hydroxymethyl)aminomethane] at about pH 8, 0.6 mM NAD, 0.1 mM glucose 6-phosphate, and approximately 1% Bio-Ban, as a bacteriostat. The sample is injected into the stream through the injection port 42 and is carried by the flowing stream to the solubilization stage 44. The solubilization stage comprises immobilized phenobarbital antibody, immobilized according to Example 3 and saturated with phenobarbital-glucose-6-phosphate dehydrogenase (GPD) complex.

As the stream and sample flow through the solubilization stage the phenobarbital of the sample competitively binds with the antibody thereby releasing phenobarbital-GPD complex into the stream. The phenobarbital-GPD complex in the stream flows from the solubilization stage into the conversion stage 48. The conversion stage comprises a few feet of chemically resistant tubing to delay the arrival of the phenobarbital-GPD complex at the detection stage for a sufficient period of time to allow enzyme magnification of the glucose-6-phosphate contained in the flowing stream. The flow rate throughout the procedure is about 1 ml per minute.

After leaving the conversion stage the phenobarbital-GPD complex flows into the detection stage. In this embodiment the detection stage is a spectrophotometric signal detector calibrated for a 340 nm absorbance measurement. The Model 300 N Micro-Sample Spectrophotometer equipped with a thermally regulated flow cell from Gilford Instrument Labs., Inc., is suitable for the detection of the phenobarbital-GPD complex. The signal absorbance generated by the phenobarbital-GPD complex is directly related to the quantity of phenobarbital contained in the original sample. Typically phenobarbital concentrations are on the order of 10 to 75 mg per liter of sample.

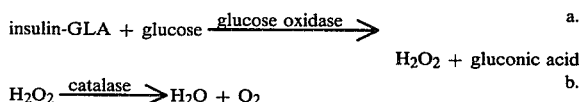
EXAMPLE 11

A 0.1 ml sample containing an unknown quantity of insulin is injected into the flowing aqueous buffer stream according to the apparatus shown in FIG. 3. The sample 64 is contaminated with approximately 20-100 mg of free glucose. The stream flows at a rate of approximately 10 ml per hour, and contains 55 mM THAM buffer, [tris(hydroxymethyl)aminomethane] and has about 1% Bio-Ban bacteriostat about pH 8. After injection of the sample into the flowing stream at the injection port 70, the sample is carried into a solubilization stage 72 by the stream.

The solubilization stage comprises an immobilized insulin antibody, prepared according to Example 1. The insulin antibody is saturated with insulin-glucoamylase (GLA) complex. The solubilization stage comprises a cartridge column containing approximately 40 ml total volume. As the stream and sample flow through the solubilization stage, the free insulin in the sample competes with the insulin-GLA complex and liberates insulin-GLA complex into the flowing stream.

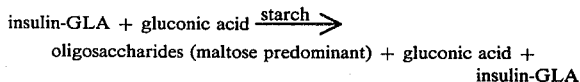
The stream and complex then flow into the conversion stage 74. The first stage within the conversion stage is the scavenger stage 76. In the scavenger stage, Reaction 1 takes place as follows:

1. Scavenger Stage



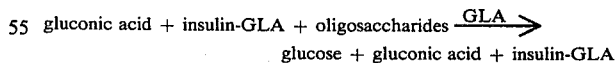
The glucose free stream and sample containing the insulin-GLA complex then flows into a substrate stage 78. In the substrate stage, Reaction 2 takes place:

2. Substrate Stage



The stream containing the GLA generated oligosaccharides flows into the glucose generating stage 80. In the glucose generating stage, Reaction 3 takes place:

3. Glucose Generating Stage



The generated glucose from the sample in the stream flows into hydrogen peroxide generating stage 82 wherein Reaction 4 takes place, and then into the detection stage 84 wherein Reaction 5 takes place:

4. Hydrogen Peroxide Generating Stage

